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Shape changes of erythrocytes during blood clot contraction and the structure of polyhedrocytes

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Polyhedral erythrocytes, named polyhedrocytes, are formed in contracted blood clots and thrombi, as a result of compression by activated contractile platelets pulling on fibrin. This deformation was shown to be mechanical in nature and polyhedrocytes were characterized using light and electron microscopy. Through three-dimensional reconstruction, we quantified the geometry of biconcave, intermediate, and polyhedral erythrocytes within contracting blood clots. During compression, erythrocytes became less oblate and more prolate than the biconcave cells and largely corresponded to convex, irregular polyhedra with a total number of faces ranging from 10 to 16. Faces were polygons with 3 to 6 sides. The majority of the faces were quadrilaterals, though not all sides were straight and not all faces were flat. There were no changes in the surface area or volume. These results describe the gradual natural deformation of erythrocytes as a part of compaction into a tightly packed array that is an important but understudied component of mature blood clots and thrombi.

Erythrocytes were commonly believed to be passive bystanders in the processes involved in blood clotting, but recent studies revealed that they play a more influential role in both hemostasis and thrombosis^{1,2}. Besides their indirect rheological effects, including marginalization of platelets in the blood flow, erythrocytes have the ability to directly modulate thrombin generation, fibrin formation, structure^{3,4}, and the viscoelastic properties of the clot^{3,5,6}. In particular, the incorporation of erythrocytes into the clot has been shown to determine the clot size⁷ and result in a hematocrit-dependent reduced extent of clot contraction (aka clot retraction)⁵. Clot contraction, or the volume shrinkage of the clot, occurs when activated platelets pull on the fibrin network^{8,9}. This results in the compaction of erythrocytes to the core of the clots and redistribution of platelets and fibrin toward the outside of the clot¹⁰. The erythrocytes amassed in the core of the contracting clot undergo a shape transformation from their native biconcave shape to that of polyhedra, hence named polyhedrocytes¹⁰. The presence of polyhedrocytes in the core of the clot reduce clot permeability and consequently are thought to play a role in hemostasis¹⁰. The reduced extent of clot contraction, implying a larger volume and a less compact structure, may aggravate arterial and venous thrombosis due to increased obstructiveness and perhaps propensity of a thrombus to rupture and embolize^{11,12}.

This remarkable polyhedral shape of erythrocytes, first described by Gottlob *et al.*¹³ and rediscovered later^{10,12,14,15}, is a natural morphological form of erythrocytes in addition to echinocytes, acanthocytes, spherocytocytes, ovalocytes, elliptocytes, stomatocytes, and more¹⁶. From a more general perspective, polyhedra have been observed in nature¹⁷ and in non-biological materials^{18,19} since before the time of Plato in a variety of plant and animal cells, viruses, tissues, foams and metals²⁰. It has been shown that some polyhedral shapes allow for the minimization of interface area between cells²¹, the minimization of potential energy in the system¹⁷, and the stabilization of the membrane skeleton²². It is biologically important that polyhedrocytes are able to minimize the space between cells, due to more efficient packing, which helps to create an impermeable seal at the site of vessel injury to prevent bleeding^{10,23}. Polyhedrocytes formed intravitaly have been observed in clots and thrombi obtained from human and murine samples^{10,12,23–25}.

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